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Fungal Contamination of Fermented *Prosopis africana* (Okpehe) and Toxicity screening of the Crude Extracts in Albino Rats (*Rattus novergicus*)

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ABSTRACT

"Okpehe", fermented African mesquite bean (Prosopis africana) is a popular seasoning among the Igala speaking of Kogi State, Nigeria. Ten samples of okpehe from different sources in and around Anyigba, Dekina Local Government of Kogi State, Nigeria were analysed for fungal contamination. The analysis was carried out in triplicates. Nine of the ten samples were found to produce at least one fungi isolate on culture in potatoes dextrose agar medium, with a total of forty-four isolates and seven different types of fungal species. Nine (20.5%) of the isolates elaborated secondary metabolites on rice medium. Thirty healthy albino rats (170 ± 32 g) were randomised into ten groups of three animals each. Nine groups were treated p.o. with a fixed dose of 5000mg/kg (body weight) of the crude extracts of the secondary metabolites produced. The tenth group served as control and the animal received equal aliquot of the vehicle (normal saline). After fourteen days of observation, neither pharmacotoxic signs nor mortality was was observed in the test animals. This shows that although the okpehe consumed in Anyigba and its environs contains residual fungi contamination from the fermentation process, may not pose any risk due to mycotoxicoses in consumers.

Keywords: Okpehe**,** African mesquite bean, Igala, Nigeria**,** mycotoxicoses.

INTRODUCTION

Prosopis africana is a Leguminosae belonging to the family of Fabaceae. It reaches 4-20m in height; has an open crown and slightly rounded buttresses; bark is very dark, scaly, slash, orange to red-brown with white streaks [1]. It is mostly found growing in the savanna regions of Western Africa. In many areas, its fermented seeds are used as a food condiment [2]. One of such product of *Prosopis africana* is okpehe , a fermented food flavouring condiment most popular among the Igala people of Kogi State Nigeria.

Several indigenous research studies have been carried out on the production of fermented condiments from various local legume and non-legume seeds [3, 4, 5, 6] (Eka, 1980; Odunfa, 1981a,b; Barber and Achinewhu, 1992; Omafuvbe et al., 2000; 2002), the specific roles of the fermenting bacterial flora on the fermenting / fermented condiment(s) [7], and nutritional values of the condiment [1]. The main objective of the present study however, is to determine residual

fungal contamination of okpehe sold in Anyigba and to determine if there are mycotoxin associated risk with consuming okpehe.

MATERIALS AND METHODS

Samples of okpehe were purchased from different locations in Anyigba market, Agbeji and Egume in Dekina Local Government Area of Kogi State, Nigeria. The samples were labelled as A, AM_1, AM_2, AM_3, AM_4, AM_5, AM_6, AM_7, EM_1 and EM_2.

Inoculation of Samples

Each of the okpehe sample was transferred into a sterile porcelain dish in a previously fumigated inoculation hood. A forcep was used break up the okpehe lump into even consistency. Triplicate plates were inoculated for each sample. These were transferred to another previously fumigated inoculation hood and then left at room temperature. Three plates of uninoculated PDA plates were kept as controls. The plates were then observed after 48 hours for fungal growth.

Isolation of Pure Cultures

Some plates had multiple fungal colonies growing on them. Such plate colonies needed to be separated into different plates. A sterile inoculating loop was used to touch the PDA close to the colony to be separated to make it sticky. The sticky end was used to touch the colony to be separated such that some spores or mycelia stick on to the loop. This was then carefully transferred to an appropriately labelled sterile PDA plate. This was repeated until all the colonies have been transferred into separate PDA plates. The newly inoculated plates were then transferred to another previously fumigated inoculation hood together with three uninoculated plates to serve as control. The plates were then observed for fungal growth after 24 hours. The plates that still had multiple colonies growing on it were further separated until pure culture were obtained. By the same method, pure culture obtained were transferred into PDA slants for storage and further investigation.

Identification of Pure Isolates

Identification of the pure isolates were carried out using [8] and [9].

Culturing Isolates on Rice Medium

Distilled water (100 ml) was added to 250 g of rice in a 1000 ml conical flask and allowed to stand at room temperature for 24 hours for moisture equilibration. It was then autoclaved to sterility using a Prestige medical clinical autoclave. The pure isolates were inoculated onto appropriately labelled rice medium in a previously fumigated inoculation hood. The inoculated rice medium were then maintained in a Grieve laboratory oven LW 201C at about 35^0C for 21 days.

Extraction of Fungal Cultured Rice Medium

After 21 days, 750 ml of methylene chloride was added to the conical flask containing the rice culture and allowed to stand for 1 hour. The culture was then pulverised using a high speed Creston grinder. The pulverised rice culture were then poured into an Erlenmeyer flask fitted to another Erlenmeyer flask and then to a Speedevac 2 suction pump through a Buchner funnel fitted with Whatman filter paper. The filtrate was then evaporated to dryness in a Soxhlet apparatus. The residue was mixed with petroleum ether in ratio 1:15 ml and then put in a refrigerator. After 24 hours, the mixture was filtered through Whatman filter paper. The filtrate was discarded and the precipitate evaporated to dryness in Grieve laboratory oven maintained at 40^0C.

Lethality Test of the Crude Extracts

The principle employed was to administer the test limit for acute toxicity test generally considered to be 5000 mg/kg body weight [10]. Sixty healthy albino rats (170 ± 32 g) were randomised into twenty groups of three animals each. Nineteen groups were treated p.o. with a fixed dose of 5000mg/kg (body weight) of the crude extracts of the secondary metabolites produced. The twentieth group served as control and the animal received equal aliquot of the vehicle (5 ml normal saline).

RESULTS AND DISCUSSION

Fungi was isolated from okpehe sold in and around Anyigba market (Table 1), although no mouldy growth was observed in the samples used. The presence of the fungi could be due to contamination or they may be part of the fermentation process of the okpehe seeds. Seven different fungi were isolated from 9 of the 10 samples (Table 2). They include a known toxigenic mould, *A. niger,* and *S. cerevisiae, , Syncephalastrum spp., Microsporum spp., Mucor spp., Cladosporium spp.* and *Paecilomyces spp,* whose toxigenic status have not been clearly established (Table 3). There were a total of 44 isolates isolated from the opkehe samples and 11 of the isolates elaborated secondary metabolites (Table 5). The most common isolates were isolates of *A. niger,* and *S. cerevisiae*.

Aspergillus niger was found in 6 of the 10 samples with a total of 15 isolates. Four of these isolates elaborated secondary metabolites. *Aspergillus niger* is relatively harmless compared to other filamentous fungi is less likely to cause human disease than some other *Aspergillus* species. Despite this fact, there have been some medical cases that have been accounted for, such as lung infections or ear infections in patients that have a weakened immune system, or an immune system that has been impaired by a disease or medical treatment [11, 12]. *Aspergillus niger* can produce a variety of secondary metabolites, termed mycotoxins, depending upon growth conditions and the strain of the organisms. The mycotoxins include oxalic acid crystals, kojic acid, Ochratoxin A , and cyclic pentapeptides called malformins. The mycotoxins range from moderately to highly toxic in terms of acute toxicity [11, 13]. Although four of the isolates of *A. niger* elaborated mycotoxins, they were not lethal in rats. This suggests that the elaborated mycotoxins were not acutely toxic.

Saccharomyces cerevisiae was also found in 6 of the 10 samples with a total of 14 isolates. *Saccharomyces* is a commonly isolated fungi from food. It may have been part of the process of the fermentation of the okpehe sseeds and what was isolated were the residual isolates. None of the isolates elaborated secondary metabolite and so there is no mycotoxin associated risk from the presence of this fungi in the samples.

Syncephalastrum spp. was isolated from only one sample and the three isolates all elaborated secondary metabolites on rice culture. The secondary metabolites elaborated however were non-lethal to rats. This suggests that there is no mycotoxin associated risk due to *Syncephalastrum* contamination from consumption okpehe sold in and around Anyigba market. Similarly, five isolates of *Microsporum spp.* were isolated from two of the ten samples. Two of the five isolates elaborated secondary metabolites, but they were not lethal to rats.

Table 1: Type and occurrence of fungi isolates that contaminate okpehe sold in and around Anyigba market

SAMPLE	PLATE 1			PLATE 2			PLATE 3		
	ISOLATE 1	ISOLATE 2	ISOLATE 3	ISOLATE 1	ISOLATE 2	ISOLATE 3	ISOLATE 1	ISOLATE 2	ISOLATE 3
A	*S. cerevisiae*	*Mucor spp.*		*S. cerevisiae*	*Mucor spp.*		*S. cerevisiae*		
A1	*A. niger*			*A. niger*			*A. niger*	*S. cerevisiae*	
A2	*Syncephalastrum spp.*	*A. niger*	*Mucor spp.*	*Syncephalastrum spp.*	*A. niger*		*Syncephalastrum spp.*	*A. niger*	*Mucor spp.*
A3	*Microsporum spp.*			*Microsporum spp.*			*Microsporum spp.*	*A. niger*	
A4	*A. niger*			*A. niger*	*S. cerevisiae*		*A. niger*		
A5	*Cladosporium spp.*	*S. cerevisiae*		*S. cerevisiae*			*Cladosporium spp.*	*S. cerevisiae*	
A6	*Microsporum spp.*	*A. niger*	*S. cerevisiae*	*A. niger*	*S. cerevisiae*		*Microsporum spp.*	*A. niger*	*S. cerevisiae*
A7									
E1	*A. niger*	*Paecilomyces spp.*		*A. niger*					
E2	*S. cerevisiae*			*S. cerevisiae*			*S. cerevisiae*		
Control	-	-	-	-	-	-	-	-	-

A total of one isolate of *Paecilomyces spp.* and two isolates of *Cladosporium spp.* were each isolated from one of the ten samples of okpehe. Two of the samples yielded four isolates of *Mucor spp.* on culture. The isolates of these three fungi did not elaborate secondary metabolite on culture in rice medium. Thus, there is no mycotoxin-associated risk from consumption of okpehe contaminated with these fungi.

It is worthy to note that none of the sample was mouldy when it was purchased from the markets, yet they had fungal contamination. It indicates that the okpehe samples were contaminated with the spores of the fungi. Even though the fungi did not produce lethal mycotoxin, care should be taken to eliminate fungal contamination, by proper handling and storage.

Table 2: Number and Type of Fungi Isolated from Samples

SAMPLE	Total No of isolates	No of Types of Isolates
A	5	2
A1	4	2
A2	8	3
A3	4	2
A4	4	2
A5	5	2
A6	8	3
A7	0	0
E1	3	2
E2	3	1
Control	0	0

Table 3: Type and incidence of fungal contamination of okpehe sold in and around Anyigba market

S/No	Fungi	No of Isolates
1	*S. cerevisiae*	14
2	*A. niger*	15
3	*Syncephalastrum spp.*	3
4	*Microsporum spp.*	5
5	*Mucor spp.*	4
6	*Cladosporium spp.*	2
7	*Paecilomyces spp.*	1
	Total	44

Table 4: Incidence of Toxigenic fungi isolated from okpehe sold in and around Anyigba market

S/No	Fungi	No of Isolates
1	*S. cerevisiae*	14
2	*A. niger*	4*/15
3	*Syncephalastrum spp.*	3*/3
4	*Microsporum spp.*	2*/5
5	*Mucor spp.*	4
6	*Cladosporium spp.*	2
7	*Paecilomyces spp.*	1
	Total	9*/44

* = No of toxigenic fungi

Table 5: Lethality of extracts of *A. niger* in rats

Isolates	No of Test Animals	Mortality
i	3	0
ii	3	0
iii	3	0
iv	3	0

Table 6 : Lethality of extracts of *Syncephalastrum spp.* in rats

Isolates	No of Test Animals	Mortality
i	3	0
ii	3	0
iii	3	0

Table 7: Lethality of extracts of *Microsporium spp.* in rats

Isolates	No of Test Animals	Mortality
i	3	0
ii	3	0

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